"Studies on Pathological, Mycorrhizal and Phytochemical analysis of *Vigna radiata* (L.) Wilczek cultivars"

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1. Introduction:

Green gram is scientifically known as *Vigna radiata* (L.) Wilczek. A member of the leguminaceae family, it originated in India and is primarily cultivated in South East Asia. The Green gram is an annual herbaceous legume pulse crop that is self-pollinated. Mung beans are well known for their high nutritional content. They have a high protein, lipid, vitamin, and mineral content. It comprises 55% to 65% of carbs in green gram s. There is between 20% to 50% protein. In which albumin is 25% and globulin is 60%. Mung bean is main sources of dietary proteins. It is a great source of green manure because it may be cultivated as a cover crop in a rotational fashion, either before or after cereal crops. It is a wonderful green manure crops.

The crop is extensively grown in India, Pakistan, Shri Lanka, Thailand, Laos, Cambodia, South China, Philippines. It is additionally grown to a lesser extent in many parts of the Africa, United States of America and Australia. The crop accounts for nearly 16 present of India's total pulse area, making it the third most important pulse crop. States like Rajasthan, Maharashtra, Andhra Pradesh, Madhya Pradesh, and Bihar are significant producers of green gram s in the nation.

The Green gram is a frost-sensitive, warm-season plant. Tropical, subtropical, and temperate climates are all suitable for growing Green gram . The ideal temperature range for Green gram germination and growth is between 15 and 18 °C. It performs well with a variety of soil types, and the recommended pH range for soil is 6.2 to 7.2. The mung bean plant prefers short days; long days will prevent it from flowering and pod formation.

Soil is the critical component of the earth system, functioning not only for the production of food, fodder and fiber but also in the maintenance of local, regional and global environmental quality. The gradual decline in the supply of soil nutrients causing nutrient (macro and micro) imbalances due to inappropriate fertilizer applications and decline in soil organic matter content. With the decline in plant growth the partial factor productivity from applied nutrients is going down resulting in increasing costs of production.

Fungi are essential components of soil microbiota, influencing plant health, nutrient cycling, and ecosystem sustainability. However, certain fungal species can also cause diseases, affecting crop productivity and quality. Therefore, investigating the fungal communities associated with mung bean plants is crucial for sustainable agriculture. Microorganisms occur everywhere on the planet and more so in the tropics where humidity and temperature are better suited for them to grow and multiply. Outdoor microorganism spread on crop and effect on crop growth. The pathogenic fungus causes several plant diseases they reduce crop growth.

Disease is one of the major constraints in economic crop production as it causes significant losses. Green gram is also attacked by many diseases during seed germination, seed production and ripening. Over 35 fungal pathogens, few viral, bacterial, and nematode species are known to attack Green gram resulting in major yield losses (Agrawal, 1989).

The mung bean, a legume plant, fixes atmospheric nitrogen in association with Rhizobia (58–109 kg/ha). It could add large quantity of biomass (7.16 t biomass/ha) and nitrogen (30 to 251 kg/ha) in to the soil. It can meet its own requirements for the element and benefit subsequent crops additionally.

The symbiotic bacteria known as rhizobia are found in the root nodules of legumes. In ideal conditions, pulse crops can fix between 50 to 80 prescent of the nitrogen that needed for growing; the remaining nitrogen has to come from the soil or fertilizer. A large quantity of nitrogen remains in the soil after a crop is harvested. Rhizobium lives in the soil and colonizes the roots of legumes. That leads to the generation of ammonia from the atmospheric nitrogen. Legumes need specific strains of Rhizobium to carry out the nitrogen fixation process through the production of root nodules. Rhizobia strain inoculation of legumes is advantageous because it boosts crop production.

Nitrogen plays a crucial role in plant growth and development. It is required in large quantities and is considered one of the most significant nutrients for plants. It‟s widely distributed in atmosphere, lithosphere, and hydrosphere. However, the amount of nitrogen available in the soil is relatively small, and only a minute fraction of it can be directly utilized by plants. Nitrogen exists mainly as nitrate and ammonium ions in the soil, which are essential for plant nutrition. Its mobility allows for the continuous exchange of nitrogen between the atmosphere, soil, and living organisms, contributing to the overall nutrient cycle.

Robust research evidence highlights phytochemicals to have gained unprecedented recognition as “Life Span Essentials”. Phytochemicals are non-nutrient, bioactive chemical compounds that are produced by plants; and within biological systems, they act as prolific dietary antioxidant and render anticarcinogenic, antiinflammatory, cardioprotective and immunity boosting effects. About 1000 phytochemicals are known till date. Foods i.e. fruits, vegetables, legumes, beans manifest an excellent dietary vehicle to provide wide range of phytochemicals (Liu Rh, 2004).

I have selected entitled “Studies on Pathological, Mycorrhizal and Phytochemical analysis of *Vigna radiata* (L.) Wilczek cultivars”

2. Review of Literature:

2.1.1. Pathological

Mehta (2004) observed that leaf blight caused by *Macrophomina phaseolina* has been identified as an economically important disease in Rajasthan which causes considerable losses 10.8 % in grain yield to the mung bean.

Kumar *et al.* (2013) recorded Alternaria leaf spot disease caused by Alternaria alternata as one of the most economically important disease of chilli in Manipuri, India.

Tandel *et al.* (2015) reported occurrence of leaf blight disease caused by Macrophomina phaseolina in Green gram during survey and it was in serious proportion caused heavy losses around navsari, Gujarat, India.

Bhaskar (2017) evaluated Green gram and Black gram genotypes/germplasms were to identify the sources of resistance to leaf spot, powdery Mildew and Mung Bean Yellow Mosaic Virus (MYMV) diseases. Screening was done under natural field conditions. Out of sixty three Green gram entries, only one KMP-13 was moderately resistant to Cercospora leaf spot disease, three entries viz., KMP-36, KMP-39 and KMP-41 were found highly resistant to powdery mildew, ten entries viz., KMP-13,19,20,22,23,24,40,45,MLGG-8 and WGG-42 were found immune to mung bean yellow mosaic virus disease. Out of eleven Black gram entries, only one MASH-338 was moderately resistant to Corynespora leaf spot disease, four entries viz., PU-31, MASH-338, LBG-752 and MBG-1050 were found moderately resistant to powdery mildew and two entries viz., PU-31 and MASH-338 were found immune to mung bean yellow mosaic virus disease. Resistants‟ can be exploited to develop high yielding varieties of Green gram and Black gram by breeding.

Sun *et al.* (2019) studied morphological characteristics of *Fusarium oxysporum* causal agent of mung bean wilt from the collected isolates by sub-culturing at 250C for 13-15 days on PDA medium and carnation leaf agar plate CLA under 12hr light/ 12hr dark cycle. The microconidia and macroconidia produced on PDA and CLA medium were observed and measured under a light microscope.

Vani *et al.* (2019) selected mung bean samples showing distinct wilt characteristics for isolation of pathogen. The selected roots, collar region and stem were washed and sterilized with 0.1% mercuric chloride

solution in laminar air flow followed by washing 3 to 4 times with sterilized water and were placed in blotting paper to remove excess moisture. The pieces were transferred to Petri plates containing potato dextrose agar medium with the help of sterilized needle. Three to four pieces of diseased parts of mung bean were placed equidistant from each other and plates were kept at 28 + 2 0C for 7 days in an incubator for further mycelial growth. Colonies resembling *Fusarium oxysporum* were purified by single spore isolation.

Kumar *et al.* (2020) reported cercospora leaf spot disease is a critical annihilating disease of mungbean (*Vigna radiata* L.). It could be a broad disease caused by the organism *Cercospora canesens* with the decrease in trim abdicate up to 60 per cent. The disease was firstly detailed in Delhi, India and spread all over the environment. Field conditions and essential natural variables are dependable for the infection rate. Numerous of the botanical extricates and fungicides are detailed to be successful control of the disease.

Sunani *et al.* (2024) reported several key pathogens viz.Anthracnose (*Colletotrichum spp*.), Powdery Mildew (*Erysiphe polygoni*), Mungbean Yellow Mosaic Virus (MYMV), Cercospora Leaf Spot (*Cercospor acanescens*) in green gram . The chapter further explores the epidemiology of these diseases, elucidating the environmental factors influencing their prevalence. It highlights the significance of accurate symptom diagnosis for effective disease management and emphasizes the implementation of integrated approaches.

2.1.2. Mycorrhizal

Denison *et al.* (2004) reported that the rhizobial strains within species can have three dissimilar genetically determined actions. The rhizobia which are in a mutual relationship with the host plants provides nitrogen while the rhizobia which are parasitic in nature infect the legumes and do not fix nitrogen. The rhizobial strains which are non-symbiotic do not infect the legume plants. A symbiotic (mutualistic or parasitic) Rhizobium that thrives in formation of a nodule might produce numerous number of descendants. The non-symbiotic rhizobia can have better reproductive success while the chances of success are very low. Legumes which have the ability to fix very less nitrogen or no nitrogen favors mutualistic strains while the parasitic strains which use plant resources only for their own reproduction do well in association with mutualistic strains.

Poonia (2011) reported that in dry arid soils, legume plants play an important role in their sustainability. Legume have the ability to provide required amount of nitrogen for the physiological growth. In agricultural fields, intensive farming is done with the use of chemical fertilizers which are expensive as well as pose a threat to the environment. Most of the legumes are nodulated by different species of Rhizobium. It is a gram negative, fast growing bacteria and has the ability to provide nitrogen. Increasing the use of bio-fertilizers such as Rhizobium is highly recommended so as to decrease the use of chemical fertilizers as well as to save environmental degradation.

Singh *et al.* (2018) reported that the development in sustainable agriculture can be brought by the use of bio-fertilizers such as Rhizobium can decrease the use of inorganic fertilizers and also reduce the severe environmental stresses. The study also revealed the positive influence of rhizobial (Mesorhizobium) inoculant on growth characteristics, symbiotic parameters, yield and yield components, nutrient uptake and quality in chickpea. Further, stress in environmental factors such as pH, nutrient availability, temperature, herbicides and moisture have a significant influence on legume -Rhizobium symbiosis, nitrogen fixing ability of bacteria and plants.

- **Nodulation capacity of Rhizobium**

Mweetwa *et al.* (2016) carried out an investigation on nodulation, nutrient uptake and yield of common bean dual inoculated with Rhizobium tropici and Trichoderma harzanium. After 51 days of planting, the nodule effectiveness and nodulation were determined. The study revealed that soils ameliorated with inorganic N slows down the process of nodulation even in presence of abundant inorganic P. Inoculation of common bean with Rhizobia and Trichoderma either alone or in combination increases the number of nodules and effectiveness per plant but is unable to increase N and P accumulation, biomass or grain yield.

Kumari *et al.* (2017) studied on effect of salinity and seed inoculation with Rhizobium on nodulation and leghemoglobin content in fenugreek. The results revealed that the nodule growth decreased with increase in salinity. Seeds inoculated with Rhizobium ameliorated the harmful effect of salinity at all levels besides improving the fertility of soil and nodule growth by nitrogen fixation.

- **Nitrogen fixation:**

Roychowdhury *et al.* (2013) reported that the legume- rhizobia symbiosis plays a major role in the increasing cultivation of legumes as the atmospheric nitrogen fixed by the microorganisms is more than that of the nitrogen fertilizers which is bought and applied by the farmers in the agricultural fields. Thus, the symbiosis between the rhizobia and legume plants are quite economical and can improve the soil fertility and increase the crop production.

Carolin C.M. Schulte (2021). Rhizobia induce nodule formation on legume roots and differentiate into bacteroids, which catabolize plant-derived dicarboxylates to reduce atmospheric N_2 into ammonia. Despite the agricultural importance of this symbiosis, the mechanisms that govern carbon and nitrogen allocation in bacteroids and promote ammonia secretion to the plant.

2.1.3. Phytochemical

Phytochemical analysis of *Vigna radiata* (L.) Wilczek**,** commonly known as Mung bean, reveals the presence of various bioactive compounds with significant health benefits. Studies have identified flavonoids, phenols, terpenoids, alkaloids, and saponins in different parts of the plant, showcasing antioxidant, anti-inflammatory, anti-angiogenic, and antiviral activities.

Phytochemicals in *Vigna radiata* (L.) Wilczek seeds and sprouts include coumarin, alkaloids, phenols, flavonoids, terpenoids, phytosterol, and saponin. Notably, vitexin and isovitexin were isolated, showing anti-angiogenic activity (Qassim and Kadhem et al., 2020). Phytochemicals found in Vigna radiata (L.) Wilczek include flavonoids and phenols. The plant exhibits antioxidant, antidiabetic, and hypocholesterolemic properties, contributing to its nutritional and medicinal value (Kalimler et al., 2021). Vigna radiata (L.) Wilczek extract contains vitexin and isovitexin, identified phytochemicals. These compounds contribute to the antiviral activity against influenza A virus demonstrated in the study (Lo et al., 2020). Naringenin, a flavonoid precursor, enhances protein bioavailability, solubility, chlorophyll content, and antioxidant potential in Cd-stressed *Vigna radiata* (L.) Wilczek, suggesting its potential for heavy metal stress tolerance in legumes (Sharma et al., 2021).

Fazal, H., Ahmad, N. and Khan, M.A. (2011). This study emphasizes the critical importance of quality control in herbal medicine. The wide variation in extractive values, ash content, phytochemical composition, and antioxidant potential underscores the need for consistent extraction protocols and rigorous testing to ensure the efficacy and safety of herbal products. Furthermore, understanding the

chemical profile of plants can guide their appropriate use in therapeutic contexts and inform the development of standardized herbal formulations.

Objective of the Study:

1. To study the Collection of germplasm of *Vigna radiata* (L.) Wilczek from Agriculture University, farmers and local market of Kandhar Tahsil.

2. To study the fertility status of soil.

3. To study the effect of various pathogens on the growth parameters.

4. To analyse the comparative study of treated and non-treated cultivars.

5. To study the Root Mycorrhizal association of the different cultivars.

6. To study the Phytochemical analysis of different cultivars.

3. METHODOLOGY

3.1 Experiment-I (Fertility Status of Soil)

In the present investigation soil sample will be collected from Kandhar tahsil location. In Kandhar we found different types of soil. The collected soil sample will be analysed at Soil Testing Laboratory, Nanded. For conducting study on a scientific investigation, methodology it‟s very important know that fertility status of soil. Though which we will get valid and reliable information about soil health. It helps us to get a meaningful conclusion.

3.1.1 Location

The soil samples will be collected from Experimental farm Kandhar tahsil place. Maharashtra state has been divided into 9 agroclimatic zones based on rainfall, soil type and the vegetation. Kandhar location mainly falls under the arid zones. Present investigation mainly fall under medium to deep black soil and deep brown to red soil (Regur).

3.1.2 Material Required

1. Khurpi
2. Spade
3. Auger
4. Bucket
5. Sampling Bag and label

3.1.3 Procedure of collection of soil sample

The sample should be collected during fallow period. First upon field will be divided into homogeneous unit based visual observation. The sampling will do several locations on filed in zig-zag manner. First layer of soil surface will be removed with the help of spade. The auger will be drive at plough depth and we will make „V" shaped cut to a depth of 15 cm. Later on sample will be collected from same place. Likewise we will collect soil sample from 10-20 place of same field.

3.1.4 Mixing of soil sample

The collected soil sample will be mixed gently and removed foreign material like roots, stones, pebbles, gravels and plant debris. Mixed sample will be spared in rectangular shape. It will be divided into four parts. The two opposite quarters are discarded and remaining are taken for reducing soil sample. The process will repeat until desired sample size about 0.5 kg obtained.

3.1.5 Testing

The collected soil sample will be sent to testing at Soil Testing Laboratory, Nanded. Result of the investigation solely depends on accurate sampling. Hence proper method was recommended for collection of soil sampling.

3.2 Experiment-II (Field Experiment-I)

3.2.1 Land Preparation

The experimental field will be prepared by cross cultivation by tractor drawn cultivator. Stubbles of previous crop and weeds will be removed from field. The experiment will be laid out as per layout plan and plots.

3.2.2 Sample Selection

The sample will be selected from three different sources *viz*., Agricultural University, From farmers and local market of Kandhar tahsil of Nanded District. The agricultural university have very good source germplasm of green gram . They have modern cultivars that are good in crop growth and yield. Many farmers are involved in collection and conservation of germplasm. These germplasm called as farmer"s variety. Farmer"s variety is good source that shows resistance against biotic and abiotic stresses. Lastly will we visit and collect seed of Green gram from local market.

3.2.3 Metrological Data:

The metrological data will regularly obtain from cotton research station of Kandhar (If Available) or Nanded, generally the climate of Kandhar was moderate. The climate of Kandhar (Nanded) is generally dry. In this region you will find three common seasons. The rainy season begins in mid-June and lasts literally until the end of September. Then there is winter from November to February and summer from March to early June.

3.2.4 Experimental Details:

Field experiment will be carried out at Experimental Farm of Kandhar tahsil, during *kharif* 2024-2025. The details of experimental layout were as under. The fungicides will be applied as seed treatment to the healthy Green gram seeds which will be used for sowing.

1)	Design	:	Randomized Block Design
2)	Variety	:	03
3)	Replications	:	3 (Three)
4)	Plot size	:	2.70 x 3 M
5)	Spacing	:	Row to Row – 45 cm Plant to Plant – 15 cm
6)	Method of sowing	:	Hand dibbling

3.2.5 Methods of Detection

A. Pathological

All three germplasm will be screened over wide range of pathogen at naturally. Crops will observe at different stages of growth. Approximate distribution of diseases in the field, random number of damage will report. Percentage intensity of all the fungi will be recorded over these germplasm.

B. Micorrhizal

In the experimental field the roots of Green gram germplasm will be uprooted at 15 and 45 days after sowing. The observation will be recorded based on number of root nodules present on the roots of different germplasm.

3.2.6 Statistical Analysis

The data generated of all the experiments was statistically analyzed (Panse and Sukhatme, 2000) and results thereof will be interpreted.

3.3 Experiment-III (Comparative Study of Treated and Non-Treated Germplasm)

3.3.1 Land Preparation

The previous year crop debris will be removed from experimental land. Land will be prepared with tractor by ploughing, harrowing and levelling.

3.3.2 Sample Selection

The sample will be selected from three different sources *viz*., Agricultural University, From farmers and local market of Kandhar tahsil of Nanded District.

3.3.3 Metrological Data:

The weather data will be recorded and data will be collected from CRS, Nanded.

3.3.4 Experimental Details:

Field experiment will be carried out at Experimental Farm of Kandhar tahsil, during *kharif* 2024-2025. Both Treated and Non-Treated plot will be sown simultaneously adjacent to each other.

1)	Design	:	Randomized Block Design
2)	Variety	:	03
3)	Treatment	:	02 (Treated and Non-Treated)
3)	Replications	:	3 (Three)
4)	Plot size	:	2.70 x 3 M
5)	Spacing	:	Row to Row – 45 cm Plant to Plant – 15 cm
6)	Method of sowing	:	Hand dibbling

3.3.5 Analysis

In the present investigation two trail of germplasm (Treated and Non-Treated) will be taking under observation in the prospective of Pathological and Mycorrhizal aspect. It will be analyzed the effect of pathogen over the both plots. It will be analyzed which treatment are better for plant growth.

3.4 Experiment- V (Phytochemical Analysis)

3.4.1 Materials and methods

3.4.1. I) Collection, Identification and Authentication plant material.

The seeds were collected from local farmer from Different village Kandar Tahsil District Nanded Maharashtra state, India. The identification and authentication of *Vigna radiata (mung bean)* was carried out using species description given by in regional Flora. The seeds were manually dissected to separate pods and seeds. The seeds dried and ground to fine powder using a Wiley mill. The powdered samples were stored in air tight screw cap bottles at room temperature till further use.

3.4.1. II) Preparation of sample extract

The powdered samples were subjected to extraction with water and 80% methanol (Sultana *et al*., 2009). About 10 g of powder was added in 50 mL of respective solvent and allowed for shaking (24h, 150 rpm, 30°C) (Ghane *et al*.,2018) on an orbital shaker. All the extracts were then centrifuged to remove debris. The supernatant was used for phytochemical investigation.

3.4.1. III) Proximate composition analysis

The proximate composition such as ash, moisture content, total carbohydrate, crude protein and amino acid content was analyzed from seeds powder. The ash content was determined by igniting the oven-dried samples in the muffle furnace at 600°C for 3 hrs. (AOAC, 2000). The moisture content was estimated by using the official method described by AOAC, (2000). The total carbohydrate content was estimated using Anthrone method (Zubaidah *et al*., 2018). The crude protein content was estimated by using modified Lowry method (Shen *et al*., 2013).

3.4.1 IV) Mineral content analysis

A total of 500 mg of *Vigna* seed flour per sample was acid digested with a mixture of 10 mL concentrated nitric acid, 4 mL of 60% perchloric acid, and 1 mL of concentrated sulfuric acid. The digested material was diluted with 50 mL of deionized water and filtered through Whatman filter paper No. 42. The final volume was made up to 100 mL in a volumetric flask with deionized water and used for mineral analysis. The minerals content were analyzed using an atomic absorption spectrophotometer (Shimadzu, Kyoto, Japan) (Issac and Johnson 1975), whereas the phosphorus content in the tri-acid digested extract was determined calorimetrically (Dickman and Bray 1940)

3.4.2 Phytochemical Investigation

3.4.2 I) Total phenolic content (TPC)

TPC was determined by using Folin-Ciocalteu method (Singleton *et al*., 1999; Chang *et al*., 2002). Accordingly, 200 µl of extract (mg/ml) was added to 1 ml of Folin-Ciocalteu reagent and mixed thoroughly. After 5 min of incubation, 0.8 ml sodium carbonate (7.5% w/v) was added and allowed to stand for 60 min at room temperature. The absorbance was measured at 765 nm using UV-Vi‟s spectrophotometer (Shimadzu, Japan). Gallic acid was used as a standard for plotting the calibration curve (20-200 µg/ml). The total phenolic content was expressed as gram s of gallic acid equivalent per 100 gram (GAE/100g) of the plant material.

3.4.2 II) Total flavonoid content (TFC)

TFC was estimated by aluminum chloride colorimetric method (Sakanaka *et al*., 2005). To 200 µl (mg/ml) extract 1.25 ml distilled water and 75 µl of 5% (w/v) sodium nitrite solution was added and incubated for 6 min. To this, 150 µl of 10% (w/v) Aluminium trichloride solution was added and the reaction mixture was incubated at room temperature for 5 min. Then the absorbance was measured at 510 nm immediately after addition of 0.5 ml of 1M sodium hydroxide solution. The amount of TFC was expressed as g Rutin equivalent per 100 gram (RE/100g) of the plant material.

3.4.2 III) Total alkaloids content (TAC)

The plant extract (1 ml) was treated with 1 ml of 2 N HCl and filtered. This solution was transferred to a separating funnel and to this 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. Then the mixture was shaken with chloroform by vigorous shaking and collected in a 10 ml volumetric flask and diluted with chloroform. A set of reference standard solutions of colchicine was prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV-Vis spectrophotometer. The total alkaloid content was expressed as mg colchicine equivalent per 100 gram (CoE/100g) of the plant material (Fazel *et al*., 2008).

3.4.2 IV) Total tannin content (TTC)

TTC was quantified by Vanillin-HCl method (Bhat *et al*., 2007). Briefly, 200 µl of the sample extract or tannic acid standard solution (50-300 µg/ml) was treated with 1 ml of reagent mixture (4% vanillin in methanol and 8% concentrated HCl in methanol, 1:1 ratio). The color developed was read after 20 min of incubation at room temperature at 500 nm. TTC was expressed as mg TAE/100g of the plant material.

3.4.3 Antioxidant activity

3.4.3 I) DPPH radical scavenging activity

The DPPH free radical scavenging activity of the extracts was determined as described by Lee *et al*., (2003). The DPPH scavenging ability as percentage was calculated as: DPPH scavenging ability = (A Control – A Sample) × 100

3.4.3 II) Phosphomolybdenum assay

Total antioxidant activity of extract was estimated using phosphomolybdenum method as described by Prieto *et al*., (1999). Aliquot of plant extract (500 µl) was mixed with 1 ml reagent (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The vials were capped and incubated in a water bath at 95°C for 90 min. After cooling the samples, absorbance was taken at 695 nm against a blank. A calibration curve was plotted using the standard ascorbic acid (20-120 µg/ml). Total antioxidant activity was expressed as g ascorbic acid equivalent per 100 gram s (AAE/100g) of the plant material.

3.4.4 GC-MS analysis

3.4.4 I) Sample preparation

Ten gram s of seed powder was subjected to extraction using the Soxhlet apparatus. The powder was placed into a thimble and extracted with 100 ml of ethyl acetate. The polarity of ethyl acetate was elevated by the addition of 1 ml of conc. HCl. The process of extraction continued for 3 hr. at a constant temperature (77°C). Finally, the volume of the filtered extract was adjusted to 100 ml and stored in the refrigerator at 4°C for less than a week for further use.

3.4.4 II) Chromatographic conditions and identification of compounds

GC-MS analysis was carried out using the Shimadzu model QP-2010 with a nonpolar 60 M RTX 5MS column. Helium was used as the carrier gas and the temperature program ming was set with the initial oven temperature at 40°C and held for 3 min and the final temperature of the oven was 480°C with a rate at 10°C. Two microliters sample was injected using a split less mode. Mass spectra were recorded over the 35-650 amu range with electron impact ionization energy of 70 eV. The total running time for a sample was 45 min. Relative quantitative determinations were made by relating respective peak areas to the total ion chromatogram (TIC). Unknown components were compared with known mass spectra of the National Institute of Standards (NIST) for molecular identification. The name, molecular weight, retention time, and peak area of the test material were ascertained.

3.4.4 III) Fourier transforms infrared spectrophotometer (FTIR) analysis

The Fourier Transform Infrared Spectrophotometer (FTIR) analysis was carried out using FTIR-7600 spectrophotometer (Lambda Scientific Pty Ltd, Australia). The methanol extract of *V. trilobata* seeds was loaded on the 100 mg of KBr pellet after hydraulic press. The pellets were mounted in an FTIR spectrometer (Lambda, FTIR-7600). The scan range was set within the electromagnetic spectrum of the infrared region of 5000 to 400 cm^{-1}. The absorption is written in terms of wavenumbers (cm^{-1}).

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Research Student — Research Guide

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